

IN THE CLAIMS:

The following listing will replace all previous versions and listings of claims in the present application.

1-38. (Canceled)

39. (Currently amended) A method of ~~inducing somatic differentiation of human embryonic stem (hES) cells *in vitro* into~~ producing human neural progenitor cells from human embryonic stem (hES) cells *in vitro*, ~~wherein said neural progenitor cells are capable of further differentiation to a cell selected from a group consisting of neurons, oligodendrocytes and an astrocytes,~~ said method comprising:

obtaining undifferentiated pluripotent hES cells; and

~~culturing the hES cells under a controlled differentiating condition which is non-permissive for stem cell renewal, does not kill cells or induce unidirectional differentiation toward extraembryonic lineages to induce somatic differentiation of the hES cells~~

culturing the cells in the presence of serum free medium supplemented with growth factors which include epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF), thereby obtaining neural progenitor cells, wherein said neural progenitor cells are capable of further differentiation into neurons, into oligodendrocytes, and into astrocytes, and wherein said neural progenitor cells express polysialated N-CAM, nestin, vimentin and the transcription factor Pax-6.

40-43. (Canceled)

44. (Previously presented) The method according to claim 39 wherein said undifferentiated pluripotent hES cells are prepared according to a method comprising:

obtaining an *in vitro* fertilised human embryo and growing the embryo to a blastocyst stage of development;

removing inner cells mass (ICM) cells from the embryo;

culturing ICM cells under conditions which do not induce extraembryonic differentiation and cell death, and promote proliferation of undifferentiated stem cells; and

recovering stem cells.

45. (Currently amended) The method according to claim 44 wherein the method for preparing said undifferentiated pluripotent hES cells is further characterized by ~~are prepared further comprising:~~

culturing the ICM cells on a fibroblast feeder layer to promote proliferation of embryonic stem cells prior to recovering the stem cells from the feeder layer, wherein the fibroblast feeder cells are arrested in their growth.

replating the stem cells from the fibroblast feeder layer onto another fibroblast feeder layer; and

culturing the stem cells for a period sufficient to promote proliferation of morphologically undifferentiated stem cells.

46. (Currently amended) The method according to claim 39, ~~wherein~~ further comprising, prior to the culturing in the presence of serum free media supplemented with growth factors, the conditions for inducing somatic differentiation of stem cells are selected from any one of the following including:

culturing the undifferentiated stem cells for prolonged periods and at high density on a fibroblast feeder cell layer to induce differentiation;

culturing the undifferentiated stem cells in serum free media;

culturing the undifferentiated stem cells on a differentiation inducing fibroblast feeder layer and wherein said fibroblast feeder layer does not induce extra embryonic differentiation and cell death;

culturing the undifferentiated stem cells to a high density in monolayer or on semi-permeable membranes so as to create structures mimicking the postimplantation phase of human development; or

culturing the undifferentiated stem cells in the presence of a chemical differentiation factor selected from the group including bone morphogenic protein-2 or antagonists thereof.

47-50. (Canceled)

51. (Currently amended) A method of inducing somatic differentiation of neural progenitors ~~to somatic cells~~, said method comprising:

obtaining a source of neural progenitor cells derived from human pluripotent embryonic stem cells *in vitro*, wherein the neural progenitor cells are capable of further differentiation ~~to a cell selected from the group consisting of~~ into neurons, into oligodendrocytes and into astrocytes, and wherein said neural progenitor cells express polysialated N-CAM, nestin, vimentin and the transcription factor Pax-6;

culturing the neural progenitor cells on an adhesive substrate in the presence of a serum free media and growth factors; and

inducing the neural progenitor cells to differentiate by withdrawal of the growth factors.

52-55. (Canceled)

56. (Currently amended) A method of inducing somatic differentiation of neural progenitors ~~to somatic cells, wherein said progenitors are derived from human pluripotent embryonic stem cells *in vitro*~~, said method comprising:

obtaining a source of neural progenitor cells, wherein said neural progenitor cells are derived from human pluripotent embryonic stem cells *in vitro*, ~~and~~ are capable of further differentiation ~~into a cell selected from the group consisting of~~ into oligodendrocytes and into astrocytes, and wherein said neural progenitor cells express polysialated N-CAM, nestin, vimentin and the transcription factor Pax-6; and

culturing the neural progenitor cells on an adhesive substrate which comprises poly-D-lysine and laminin in the presence of a serum free media, ~~and inducing to induce somatic differentiation of the neural progenitor cells to differentiate to somatic cells under conditions which favor somatic differentiation.~~

57. (Currently amended) The method according to claim 56 wherein after culturing the neural progenitor cells on an adhesive substrate in the presence of a serum free media, the cells are further cultured in the presence of retinoic acid.

58. (Currently amended) The method according to claim 56 or 57 wherein ~~said somatic cells~~ are the neural progenitor cells differentiate into neurons.

59. (Canceled)

60. (Currently amended) A method of inducing somatic differentiation of neural progenitors ~~to somatic cells~~, said method comprising:

obtaining a source of neural progenitor cells, wherein said neural progenitor cells are derived from human pluripotent embryonic stem cells *in vitro*, ~~and are capable of further differentiation into a cell selected from the group consisting of neurons, into oligodendrocytes and into astrocytes~~, and wherein said neural progenitor cells express polysialated N-CAM, nestin, vimentin and the transcription factor Pax-6;

culturing the neural progenitor cells in serum free medium in the presence of PDGF-AA and bFGF; and

plating the neural progenitor cells on an adhesive substrate which comprises poly-D-lysine and fibronectin, in serum free medium without PDGF-AA or bFGF, wherein the neural progenitor cells are cultured before and after plating on poly-D-lysine and fibronectin in serum free medium in the presence of PDGF-AA and bFGF; and thereby inducing somatic differentiation of the neural progenitor cells to differentiate to somatic cells under conditions which favor somatic differentiation.

61. (Currently amended) ~~The method according to claim 60 wherein the progenitor cells are cultured after plating on said adhesive substrate in the presence of PDGF-AA, basic FGF and EGF~~ A method of inducing somatic differentiation of neural progenitors, said method comprising:

obtaining a source of neural progenitor cells, wherein said neural progenitor cells are derived from human pluripotent embryonic stem cells *in vitro*, are capable of further differentiation into neurons, into oligodendrocytes and into astrocytes, and wherein said neural progenitor cells express polysialated N-CAM, nestin, vimentin and the transcription factor Pax-6;

culturing the neural progenitor cells in serum free medium in the presence of PDGF-AA and bFGF;

plating the neural progenitor cells on an adhesive substrate which comprises poly-D-lysine and fibronectin;

culturing the neural progenitor cells in serum free medium in the presence of PDGF-AA, bFGF and T3; and

inducing somatic differentiation of the neural progenitor cells by withdrawing PDGF-AA and bFGF in the medium.

62. (Canceled)

63. (Currently amended) The method according to claim 60 or 61 wherein ~~said somatic cells induced are~~ said neural progenitor differentiate into oligodendrocytes or astrocytes.

64. (Currently amended) A method of producing an enriched preparation of human pluripotent ES cell derived neural progenitor cells ~~wherein the neural progenitor cells are capable of further differentiation to a cell selected from the group consisting of neurons, oligodendrocytes and astrocytes,~~ said method comprising:

~~obtaining undifferentiated pluripotent human embryonic stem cells comprising obtaining an *in vitro* fertilised human embryo and growing the embryo to a blastocyst stage of development; removing inner cells mass (ICM) cells from the embryo; culturing ICM cells under conditions which do not induce extraembryonic differentiation and cell death, and promote proliferation of undifferentiated stem cells; recovering undifferentiated stem cells;~~

~~inducing somatic differentiation of the undifferentiated embryonic stem cells to the neural progenitor cells by providing differentiating conditions which are non-permissive for stem cell renewal, do not kill cells or induce unidirectional differentiation toward extraembryonic lineages~~

culturing the cells in the presence of serum free medium supplemented with growth factors which include epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF), thereby obtaining neural progenitor cells, wherein said neural progenitor cells are capable of further differentiation into neurons, into oligodendrocytes, and into astrocytes, and wherein said neural progenitor cells express polysialated N-CAM, nestin, vimentin and the transcription factor Pax-6;

~~identifying the neural progenitor cells by expressed markers selected from the group consisting of polysialyated N-CAM, an intermediate filament protein, and the transcription factor Pax-6; and~~

culturing the neural progenitor cells to promote proliferation and propagation.

65-66. (Canceled)

67. (Currently amended) The method according to claim ~~64~~ 66 including further culturing to eliminate non-neural cells, said further culturing comprising selective culturing in serum free media including DMEM/F12 supplemented with growth factors.

68. (Currently amended) The method according to claim 67 wherein the further culturing includes the transfer of undifferentiated ES cell clumps into serum free medium comprised of DMEM/F12 supplemented with B27, bFGF and EGF₁ and cultivation of the resulting neural progenitors as spheres or monolayers.

69-85. (Canceled)

86. (Previously presented) The method according to claim 58 wherein said neurons are mature neurons.

87-93. (Canceled)

94. (Currently amended) The method of claim ~~89~~39 or 64, wherein the neural progenitor cells are cultured as monolayers or spheres.

95. (Canceled)

96-99. (Canceled)

100. (New) The method of claim 39, further comprising, prior to the culturing in the presence of serum free media supplemented with growth factors, culturing the undifferentiated stem cells for prolonged periods and at high density to induce differentiation.

101. (New) The method of claim 39, further comprising, prior to the culturing in the presence of serum free media supplemented with growth factors, culturing the undifferentiated stem cells

on a fibroblast feeder layer wherein said fibroblast feeder layer does not induce extra embryonic differentiation and cell death.

102. (New) The method of claim 64, further comprising, prior to the culturing in the presence of serum free media supplemented with growth factors,

culturing the undifferentiated stem cells for prolonged periods and at high density to induce differentiation;

culturing the undifferentiated stem cells in serum free media;

culturing the undifferentiated stem cells on a fibroblast feeder layer and wherein said fibroblast feeder layer does not induce extra embryonic differentiation and cell death;

culturing the undifferentiated stem cells to a high density in monolayer or on semi-permeable membranes so as to create structures mimicking the postimplantation phase of human development; or

culturing the undifferentiated stem cells in the presence of a chemical differentiation factor selected from the group including bone morphogenic protein-2 or antagonists thereof.

103. (New) The method of claim 64, further comprising, prior to the culturing in the presence of serum free media supplemented with growth factors, culturing the undifferentiated stem cells for prolonged periods and at high density to induce differentiation.

104. (New) The method of claim 64, further comprising, prior to the culturing in the presence of serum free media supplemented with growth factors, culturing the undifferentiated stem cells on a fibroblast feeder layer wherein said fibroblast feeder layer does not induce extra embryonic differentiation and cell death.